Molecular Biology
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Module - 03
Basics of Biomolecules
Lecture-13 Biomolecules (Part 3: RNA)

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT Guwahati and what we were discussing, we were discussing about the different types of biomolecules in the course molecular biology. Both of these biomolecules have very crucial roles in running the different types of metabolisms or they are contributing in one or other way into the different types of molecular biology processes. So, what we have discussed, we have discussed about the DNA. In the previous lecture, we have discussed about the DNA and DNA is the crucial biomolecule which is required for the genetic information. So, it carries the information from the one generation to another generations and then we have also discussed that the DNA which is going to be involved into a process called replications and the transcriptions.

So, with the help of the transcription, the DNA is actually going to synthesize the RNA and this RNA is then actually going to participate into the reaction of the protein synthesis in a process which is known as translations. So, the enzyme which is actually going to synthesize the RNA from the DNA is known as the RNA polymerase. So, RNA polymerase is actually going to read the DNA and it is actually going to synthesize the RNA and this process is called as transcription and then RNA is actually going to participate into a series of events which is known as translation to synthesize the protein. And so, in today's lecture, we are going to discuss the properties of the RNA and the structure of the RNA and how you can be able to isolate the RNA from the cell and how you can be able to characterize the RNA so that and estimate the RNA and so on.

So, why it is important for us to understand the RNA because the RNA is mainly been responsible for synthesis of the protein. So, if you want to do an experiment related to the expression studies and other kinds of studies, then you are supposed to study the RNA. Now, when we talk about the RNA, we are actually going to talk about the three different types of RNA. So, we have three different types of RNA, we have the transfer RNA or commonly known as tRNA, so this is called as transfer RNA. Then we also have the ribosomal RNA or it is called rRNA and we also have the messenger RNA or it is called as mRNA.

The mRNA is actually going to provide the message or actually it is going to provide the informations in which sequence we are actually going to add the amino acids, so it

actually going to provide the information of the synthesis which means which amino acid I should add like that kind of information. So, for example, if I want to start writing a letter, I have to first know what is the sentence. So, if I know the sentences, then my brain is actually going to read that sentence and that sentence is nothing but this messenger RNA. And then I am going to bring A, B, C, D like that, so that A, B, C, D information is this. Now you are actually going to read the help of the transfer RNA and the ribosomal RNA.

So, transfer RNA is actually going to bring the amino acids in the same sequence what the sequence is given here, right. So, if it says you bring the alanine, right, it is actually going to bring the alanine. If it is going to say the methionine, then it is actually going to bring the methionine. Then who will bring the, so this is actually going to bring the amino acids. Then these amino acids are actually going to be joined by the ribosomal RNA, right.

And you know their amino acids are actually going to join by a bond which is called as peptide bond. So, basically the job of the ribosomal RNA is to form the peptide bond between the A and B, right. And that is how it is actually going to start synthesizing the protein molecules, right. So, these are the some basic or the brief overview of that function of these RNA species. One it is actually going to provide the message.

So, it is actually going to provide the information of synthesis, right. In what sequence I should add the amino acids and also going to provide which amino acids. And then the transfer RNA is actually going to bring that particular RNA and the ribosomal RNA is actually going to collect the information from the transfer RNA and messenger RNA and that is how it is actually going to join the amino acids by the help of a peptide bond. And that is how you are actually going to have the sequence and it is actually going to have the information, okay. So, this is just alphabets actually.

There is no amino acid with the B, right and so on. So, today in today's lecture, we are not going to cover the structure of transfer RNA or the ribosomal RNA. In today's lecture, we will only focus on to the messenger RNA. We will take up the structure of transfer RNA and ribosomal RNA when we are actually going to discuss about the translation because all of these three messenger, all of these three RNA species are actually going to participate actively into the translation process. So, we are only going to focus today, in today's lecture, we are only going to focus on to the messenger RNA.

In our subsequent lecture, we will focus on to the transfer RNA and ribosomal RNA when we are going to discuss about the translation. So, we will talk about the structure of the messenger RNA. So, messenger RNA is actually as I said, it should have the

information of the synthesis, right. So, it is going to provide the information of the synthesis of protein, which it actually going to take up from the DNA, right. So, that information is originally being present in DNA, but that information will then going to be taken up by the messenger RNA.

And so, messenger RNA is having the three distinct part. It is actually going to have the, so this is the 5 prime end and this is actually the 3 prime end, okay. And remember that we have discussed in detail about the RNA structures when we or the composition of the RNA when we are talking about the DNA, right. So, RNA is actually also a poly nucleotide molecules, RNA is single stranded in majority of the cases and RNA is also going to have the phosphodiester linkages and phosphate and black bone. So, RNA is also going to be made up of the sugar, phosphate and base, right.

And as far as the base is concerned the RNA is actually going to have the A, G and U and C, it does not have the T, right. So, there is no T present, okay. So, T is absent in the case of RNA, whereas the T is present in the DNA. Instead of T, it is actually going to have the uracil, okay. And that is the basic difference between the RNA and the DNA.

Other than that, it is going to be single stranded. So, there will be extensive secondary structures what are going to be present in the RNA species. And as far as the structure is concerned the RNA is going to have the 5 prime cap. So, this is the cap which is actually going to be protect the RNA sequences because RNAs are very, very susceptible for the RNA molecules. And then it is actually going to have the 5 prime UTR.

So, 5 prime UTR is a place which is actually going to provide the docking site for the RNA polymerase. In detail actually how the RNA polymerase is going to sit and how it is actually going to recognize the promoter regions and all that, that actually we are going to discuss when we are going to talk about the translation. So, in the 5 prime UTR you are going to have the promoter, right. And there is a definite composition of the promoter. So, it is going to have the data box, it is going to have the minus 10 region, minus 35 regions and so on.

So, all that I think can be discussed when we were discussing about the transcription and translation. So, promoter can be of strong promoter or it can be of weak promoters. So, promoters are actually going to provide a docking site for the translations initiation site. So, it is going to provide the translation initiation site and it is going to allow the sitting of the RNA polymerase. And so, it is going to provide the docking site for the protein synthesis.

So, it is going to provide a docking site for the ribosomal machinery. And this was

going to be a promoter. So, it is actually going to be a strong promoter or the weak promoter and it is actually going to provide the docking site for the translational machinery. Next to this, you are actually going to have the coding sequence. So, this is a region which is going to be a coding sequence.

So, this is the region which is going to give you the protein. So, it is actually going to provide the information in terms of the genetic code and these codes are actually going to be read by the ribosomal machinery and as well as by the tRNA and that is how it is actually going to have the help you in the synthesis of the protein. So, genetic information is encoded in the face of genetic code and each genetic code is actually going to be corresponding to the amino acids or these amino acids are then going to be added into the ribosomal machinery by the help of the peptide bond and that is how it is actually going to synthesize. And then you also have the 3 prime UTRs. So, 3 prime UTRs are actually going to be the regulatory side which actually going to provide the regulation of this whole translation process and then at the 3 prime end you are actually going to have the polyadenylation side.

So, polyadenylation is very important because polyadenylation you can actually have the addition of the ACE starting from the 50 to 200 and depending upon the polyadenylation you are actually going to decide the age of the messenger RNA because this is actually going to chewed because remember that from this side by a RNase. So, if the RNA is chewing this amino acid this messenger RNA because messenger RNA is going to be present in the cytosol inside of the nucleus. So, RNA is actually going to be synthesized by a process of transcription inside the nucleus then it is actually going to be transported outside into the cytosol and then it is actually going to provide a docking site for the protein synthesis machinery which means it is actually going to allow the assembly of the ribosomal machinery like the small subunit and the large subunit and that will happen on to the 5 prime UTR and then it is actually going to synthesize the proteins. But how long this amino is this messenger RNA is going to remain active into the cytosol that will be decided by the 3 prime poly A tails. So, this region is called as the poly A tail and this poly A tail is going to be or the ribosomal RNase which are very active within the cytosol are actually going to chew these RNA from the 5 prime, 3 prime end and the moment they are actually going to be chewing up chewing up like this they once they hit the coding sequence then they are actually going to start you know the this messenger RNA will not be useful for the synthesis or for the synthesis of the protein because now it is actually going to start synthesizing the you know the cryptid protein or the truncated proteins and that may not be good for the cell.

So, this length of this polyadenylation or length of the poly A tail will actually going to inside the edge of this particular messenger RNA or I will say the stability of this

messenger RNA within the cytosol. So, there are messenger RNA where you have the very huge number of A's and they will actually going to be remain in the cytosol for very very long time so that they will be keep expressing the protein. So, some of these messenger RNase are belonging to the housekeeping genes. For example, you have the messenger RNA for actin, myosin, LDH. So, these are the proteins which are required in a you know in a there is a huge demand of these proteins and that is why they are supposed to synthesize and since they are housekeeping genes or they are actually housekeeping proteins their level will actually going to determine the health of that particular cell and that is why they will actually going to have the huge number of poly A tail or huge number of amino acids A residues into the poly A tail.

The poly A tail is a very very interesting tool because it also provide the stability to the messenger RNA. At the other hand, it also can be a tool to purify the messenger RNA from the cytosol. So, that we can do with the help of a affinity column and that is what we are going to discuss now. So, we are going to purify the messenger RNA from the cytosol. So, there are two methods by which we can be able to isolate the messenger RNA.

One we can actually use the affinity column and we can use the affinity DT columns and the other approach is we can actually be able to use the trizone method. So, in an affinity DT column what we have is we have a linker actually and it is attached to the beads and this linker has the T residues which means it is actually has the thymine. So, when you have a thymine you know that A is always making a pair with T and G is always making a pair with C. So, utilizing this information if you have the A's what is present onto the messenger RNA. So, what you can do is you can actually be able to have the beads and you can actually be able to put the beads into the cytosol.

So, what will happen is that you are actually going to have the binding of A's which are be a part of the polyadenylation tail and it is actually going to have the messenger RNA. So, this is the messenger RNA and it is going to have this right. And ultimately what you can do is you can actually be able to do the elution and at the end what you are going to have you are going to have this eluted right. So, this is actually going to give you the complete pool of messenger RNA. This is another method which is called as triazole method where you are actually going to use the triazole and that is how you are actually going to isolate the messenger RNA.

So, both of these methods we are going to discuss how you can be able to isolate the messenger RNA from the cytosol. So, for first method, so this is the method 1 right and this is the method 2. So, for the first method what you are going to do is you are going to test grow the cells right. So, these are the target cell.

You can have also the tissue right. So, depending on what kind of material you are actually. So, if it is a tissue then you are actually going to grind the tissue. So, that it is actually going to give you the single cell suspension right. Sometime you might have to use the enzymes and other kinds of treatments.

So, that we are not discussing there. So, but if it is starting with a tissue for example, you started with liver right. So, if it is started with the liver then it has to be grind fine with the cell mortar or the homogenizers and then the liver is actually going to give you the single cell suspension and then from the single suspension you are actually going to use the same way as you are actually going to use the cell from the cell culture. So, you are going to put them into a lysis buffer. Mostly the lysis buffer contains the SDS and also contains the protein SK okay. And it also contains the sometime SDS or sometime TITON-X100 okay.

So, it is basically going to contain the detergent and the protein SK and it is also going to have the binding buffers. So, you lysed the cells under the lysis and the binding buffer and then you are going to, so this is what we have shown here right. If you have a tissue you can just do the homogenization so that you are going to have the single cell suspension and then you can incubate this. Once you incubate this it is actually going to lyse the cells and you are going to have the cell lysate okay. So, with the cell in the cell lysate you can actually you can do the spinning at for example 1000 rpm.

So, that we are actually going to remove the nucleus and then it is actually going to give you the cell lysate because the nucleus is useless because it actually going to increase the contamination. So, nucleus if you remove the nucleus you are actually going to get rid of the DNA right. And then you take the messenger RNA you put it into the binding buffer and then you are actually going to have the oligo-dt beats and as I explained the oligo-dt beats are that you are going to have the aero beats and then it is also going to have the linker that linker is actually going to have the T residues attached to it okay. This means it is these linker are actually going to have very strong and specific binding for the a residues okay. So, when you do that the messenger RNA what is present in this particular cytosol.

So, it is not specific for a particular messenger RNA it is actually be responsible for all the messenger RNA and that is how it is actually going to bind the messenger RNA. So, this is the messenger RNA. So, they will interact with each other and then you are going to do a washing with the buffer because there could be some non-effective interaction. So, you can do a washing with the buffer that washing can be done with the you know the buffer with salt right.

So, you can actually add some salt. So, that you are actually going to reduce the non-specific interactions and then you are actually going to do the elusions okay. So, once you have the pure sample you are actually going to do the elution. So, elution can be done with the so you at this step you collect the beats and then you are going to discard the thropanactin and then you are going to do the elution. So, you can add the for example you can add the poly T or you can actually add the thiamine right and then you can suspend that into the elution buffer and the elution buffer is going to allow or it is actually going to break the hydrogen bonding between the poly T tail which is attached to the beats versus the poly A tail which is present onto the messenger RNA and that is how the messenger RNA is going to be eluted and then you can actually take this pure messenger RNA for the further downstream applications like RT-PCR and you can use that for other kinds of applications. So, this is exactly what people were doing when you are actually asking them to do the COVID testing okay.

So, they were taking your saliva and other kinds of samples and then they were doing this process to isolate the messenger RNA and then they were doing the RT-PCR with the help of the primers for COVID and that is how they were saying that if it is a they were getting the amplifications of the DNA for the of the cDNA then they are actually saying that it is COVID positive. Anyway, that is separate part that anyway we are going to discuss when we are going to talk about the real-time PCR and reverse transcriptase RT-PCR and we will also going to take up how you can be able to use the RT-PCR for these kind of applications. So, this is the first method where you are actually going to use the affinity column to purify the messenger RNA from the cell lysate or the tissue. Now let us move on to the next method and the next method is called as the Trizole method. So, the Trizole method RNA isolation by the Trizole method, this RNA isolation by the Trizole method uses the Trizole which is also called as the tri reagent for the isolation of the total RNA.

Trizole is a mixture of guanidine thiocyanate and phenol which effectively dissolve the DNA, RNA and the protein on homogenization or the lysis of the tissue samples. After adding the chloroform and centrifugation the mixture separates into the three phases with the upper clear aqua phase containing the RNA, interphase containing the cell debris and the lower is the organic phase having the protein and the lipids. The next step in the extractions are the washes and the precipitation of the RNA. The first part of the protocol from the homogenized tissue in Trizole to the point of an RNA palette in 75% ethanol take less than an hour. The RNA can then be stored for long period of time at minus 20 degree Celsius.

So RNA is very stable when you are isolating with the Trizole method and putting it

into the 75% ethanol. The same protocol can be used for RNA extraction from the cell culture. So if you want to remove the DNA you can actually be able to treat the sample with DNAs and that is how it is actually going to remove the DNA part. So this is what it is actually going to say that if you have the grinded adipose tissues, for example, this is the tissue. So in the step one, you are actually going to add the reagent and that is how you are going to vertex then you are going to wait five minutes at room temperature and then it is actually going to give you the aqua phases and you are going to have the different types of buffers and what you are going to see here is that when you are going to have the phases, you are going to have two phases.

One is the RNA phase, the other one is going to be chloroform phase and in this phase you are actually going to have the protein plus lipid. Whereas in the aqua phase, you are going to have the messenger RNA and that you can actually be able to. If you transfer this aqua phase, then you can actually be able to use that by precipitation with the 75% ethanol and you can actually be able to air dry this pellet and then you dissolve this into a RNA 3 buffer and that is how you are going to have the RNA. So let us see what are the different methods or different protocols, right. So this procedure is very effective for isolating the RNA molecules of all types from 0.

1 to 15 kB in length. However, there are commercial kits that enable the simple RNA extractions using a column that binds the RNA and so on. That is anyway we have discussed, right. So what are the requirements? So first thing is you are actually requiring the trizol to require the 1.5 ml Eppendorf, you require the centrifuge, you require the chloroform, isopropanol, RNA 3 water, micropipettes and the tips and the test specimen.

So you require the either the tissue or the cell. So first step is that you are going to either take the tissue or the cell culture cells and then you are going to do the homogenization or the lysis. And once you do the vertexing and all other kind of things, then you are actually going to add the trizol and that actually is going to have the phase separations. So you are going to have the aqueous phase and then you are also going to have the organic phase. In the organic phase, you are going to have the lipid and you are going to have the protein, right. And then you collect the aqueous phase and then you add the ethanol and that is how it is going to form the pellet, the RNA pellet that you do by air dry and then you add the RNA 3 water and resolve things.

So in the step one, you are going to add the trizol reagent to the cell and incubate at room temperature for five minutes. Then you transfer the cell lysate to a 1.5 ml centrifuge tube and add 0.

2 ml of chloroform. So this is what you are going to do, right. In the step one, you are going to add the chloroform, mix it thoroughly and incubate at room temperature for five minutes. Then you centrifuge the mixture to the centrifugation at 12000 Rg for 15 minutes at 4 degree. Transfer the aqueous phase containing the total RNA to a fresh tube and precipitate the RNA by adding the 0.5 ml of isopropanol followed by incubation at room temperature for five minutes, 10 minutes. Then you centrifuge the precipitate at 12000 G for 10 minutes at 4 degree Celsius.

And then you discard the supernatant and air dry the RNA pellet for 10 minutes and resuspend in 20 microliter of RNA 3 water. Remember that this is very important and you can actually be able to either purchase the RNA 3 water from the commercial vendors or you actually can prepare the RNA 3 water in a laboratory. So it is not very difficult part. Then you perform the agarose gel electrophoresis to check the integrity of the RNA. This anyway we are going to discuss when we are going to discuss about the northern blotting.

So that time we are going to discuss about how you can be able to run the RNA gels and how you can be able to test whether the RNA quality is good or not. The RNA isolation by the tribal method is showing after adding the chloroform and centrifugation the mixture separate into three phases with the upper phase the aqueous phase containing the RNA, the interface containing the cell debris and the lower is the organic phase containing the protein and the liquid. So we have actually prepared a very small demo clips where we have actually going to show you how you can be able to isolate the RNA with the help of the tribal method and here the students have actually isolated the RNA from the bacterial cells. But you can actually be able to follow the similar steps even with the mammalian cells or the tissue. As I said you know when we are going to deal with the tissue you are actually going to homogenize the tissue so that you can get the single cell suspension.

So I hope this video or the demo video is going to be useful for you to advance your work. Today we will be learning about RNA isolations from the bacterial culture. As you can see this is a bacterial suspension already prepared. This is a suspension of the Staphylococcus aureus and we have already allocated.

So for RNA isolation we will be needing around 800 microliters. We have already allocated in the Eppendorf tubes. So to do that we need a laminar flow so that the contaminants does not get out. So as you already know we have already allocated around 800 microliters of bacterial suspension. So we will be today performing RNA isolation from this bacterial suspension. RNA isolation is a very tricky step as because it is easily degradable in the environment.

So to do that we have already given the UV for the whole hood. We have cleaned the pipettes with the 70% ethanol and all the tips and everything has been UV irradiated before use. So for RNA isolation our protocol is that RNA isolation can be done in from three simple steps. One is to homogenize the bacterial cells to take out the RNA from it, then to precipitate the RNA and then to purify the RNA. So first step we will do the homogenization of the RNA.

So to do that we will be using the tri reagent. This reagent is basically is a trizol. So trizol contains basically gonadine, thiocyanate and phenol and it actually has an, it inhibits the RNA's activity so that the RNA is not degraded in the system. So now the protocol is we will be adding around 160 microliters of trizol in the suspension culture. The cap should be put down in the laminar as always and this trizol reagent will help to homogenize as well as protect the RNA integrity in the suspension. So to homogenize this, the thing is very simple we have to pipe it in and out faster so that the bacterial cells are homogenized as you can see in this step.

I am pipetting little bit vigorously in and out. After that thing is done we will be adding chloroform around one fifth of the total volume of this. So earlier it was 800 we have added around 160 more so it is around 950 microliters. So one fifth of the volume will be adding a chloroform in that. So chloroform will do one thing it will help separating out the phases in the mixture as such.

So now we will be adding chloroform in the trizol mixture we have already done. So chloroform we have allocated in this reagent bottle. So we will be adding around one fifth of the volume which is around 32 microliters. So as I told you before chloroform will help in separating out the phases. So actually trizol is very helpful by using trizol we can separate all the three components the DNA, RNA and protein as you will see in the subsequent experiment. So after adding the chloroform what we will do is we will tilt a little bit very gently which will not be harsh.

We will tilt a little bit and then we will leave it for incubation for 2 to 3 minutes at room temperature. So I will just put it here and wait for 2 to 3 minutes. So after that we will be putting it in the centrifuge. We have to centrifuge it for 12000 rpm for 4 degree for 15 minutes.

So that the phase separation might happen. So I have already put the in the centrifuge itself and I will start it now. So it will run for 15 minutes and then we will get back. So as you can see the run is just now going to stop. So we will be taking it out and then processing it further.

So after we take out we will see the layers getting formed. So after centrifugation as you can see very clearly that there are three types of layers which are formed. This is an aqueous phase which has the RNA. A white type of layer you see this contains DNA and the pink layer if you see that contains the protein. So from trizol we can isolate all three DNA RNA and protein together.

But for today's experiment we will be doing RNA isolation. So we will be taking out the aqueous layer which is on the top. We have to be really careful not to take out the interface or the bottom layer. So we will be allocating this in the new centrifuge very very carefully. We are now allocating it the aqueous phase in the new Eppendorf tube.

So it would be you have to be very careful. So not actually you have to take it very slowly. Tilt a little bit and slowly pipette it out.

Don't just take the interface. That is the whole point. So we have taken the phase. We will be allocating it now. Still some is left so I will try to take more out of it. As you can see I am tilting it so that I will see clearly where the thing is going on. So it is always safe to not touch the interface.

So as you can see I have already allocated it and have not touched the interface. And this much RNA is more than enough for our experiment. Technically speaking we should not talk when we are doing RNA exhalation. As our when our aerosols from when we are talking might contain some type of RNAs which might degrade the RNA. So when we are doing RNA exhalation we should not talk much about it.

Now we will be adding isopropanol alcohol. This will help to precipitate the RNA from the aqueous phase. So how will we do that? We will be actually centrifuging it after adding equal volumes of isopropanol. So now we are centrifuging it for 10000 rpm for 10 minutes. So this will help out to precipitate the RNA after addition of isopropanol alcohol.

So now the centrifuge is almost complete. We will be moving to the last step of the RNA isolation which is the purification step by adding around 70% ethanol. And then again centrifuging it back. So now we have centrifuged it after the addition of isopropanol alcohol. Here as you can see the pellet is a little very less as well as it is a little bit shaky.

That might be due to the less suspension culture. I think the bacteria was not that much to give a very big thick pellet. But still the pellet is there. So now we have to very

carefully remove the isopropanol alcohol and add 70% alcohol which we have already prepared. So for the 70% alcohol preparation we have used mercury ethanol and we have used double distilled autoclave water.

Which is filtered using 0.2 micron meter membrane filters. So let us remove this isopropanol first. We have to remove very slowly so as to not take the pellet out. And if you are not sure whether the pellet will come or not you can leave it and then again take a small pipette. For example I am using a P1000 which is a grading of 100 to 1000. So for now I will leave this pipette and then I will take another pipette which is of a lower volume so that I can slowly take out the isopropanol.

So this is a pipette of 20 to 200 micrometers. So this would be a best fit for my thing now. So I will be setting it to around 100 micrometers. It is less volume and easily we can take it slowly. Now we have taken out the isopropanol.

And we will be adding 70% ethanol. So 70% ethanol we have to re-suspend the pellet. So what we have to do is we will add around 1 ml. So you can add 1 ml you can add 500 ml. This is a washing step also and purifying step also for RNA. So after you add you have to pipe it in and out a little bit so that the thing is re-suspended. And after that we will go ahead with the last centrifugation which is 10,000 rpm for 10 minutes at 4 degree centigrade.

So I am adding around 1 ml completely. And it is done. So we will go ahead with the centrifuge now. We are performing the last centrifugation step of the entire protocol. We will be doing 10,000 rpm for 10 minutes at 4 degree centigrade. And this will be helpful for washing the RNA. Now we have centrifuged the last step which was after addition of ethanol 70%. So the next step is to air dry it, take out the whole ethanol, air dry it and after air drying it for 10 to 15 minutes we will add RNA's water so that we can re-suspend the pellet in it.

So let's take out the ethanol. So after this step has been done, what we can do is because the pellet is there, we can just a little bit and let the residual ethanol come out here. Now we will keep it for air drying. Now it is after 15 minutes, the thing is fully dried. So now we will be adding the RNA's free water.

So this water we have already showed you before we have made the 70% ethanol. So we will be adding around 50 micrometers to it and then re-suspending the pellet again. So just add and re-suspend it a little bit.

Don't be too harsh. Pipe it a little bit and then just leave it. So the next step will be to

quantify this RNA. First we will quantify it using NanoDrop and secondly we will see the RNA using the gel electrophoresis. So the RNA can be stored at minus 20 degree centigrade. We should not store it at 4 degree for short time. So I will be storing this at minus 20 degree centigrade till further experiments are done in this.

Now today we will be doing RNA quantification. So this is an instrument, it is a NanoDrop machine. So this will measure the amount of RNA in one micrometer. So we will go to the nucleic acid section. As you can see in nucleic acid section there are lot of acids which can do it, can do protein and less everything.

So this is the whole template. Here you will see the concentration. This is 8 to 60 by 280 reading. A peak will come if the RNA is there. So not is set for dsDNA, means double stranded DNA. What we will do is we will set for RNA.

And now we have clicked RNA as you can see. We will proceed with the blank addition. To measure blank first what we will do as you can see here. So this is the portion where we load our sample area.

So we first clean it nicely both sides. Wipe it. This is our lint free wipe. After that we will add RNA's free water where we have already suspended in our RNA. So we will add one microliter exactly. So here addition is to be done on the red dot as you can see here.

So I will be adding directly here for RNA quantification. So now the whole sample has been loaded. I will close it and select blank as you can see here. So I will click on blank now.

So now it will adjust the blank reading first. And then after that we have to load our sample. So now the blank has set. We will load our sample after that. So to do that we have to first wipe out the RNA. So to do that we have to first wipe here again because we have already added.

And then what we have to do is take our RNA whatever we have made. Add it. And just add it. Close it and say sample. This green one is the sample. So we will just click it here. So it will measure the RNA content in the concentration of nanogram per microliter.

So as you can see RNA the concentration is 556.52. The A260 by 230 reading is 2.23 something which is a very good reading. There is no contamination. A peak, a nice peak we can see at 260. And so it is a very good amount of RNA without no contamination of

protein and DNA. So now I am sure you might have seen the demo video.

And this demo video could be very useful for you to replicate these steps in your laboratory. Although we have shown the steps with the help of the simple system like the prokaryotic system. But it can be replicated with the other type of cells also. So you can use the mammalian cells. You can use the yeast.

You can use the even the different types of tissue materials or you can actually be able to use the bacterial cells. The first step one is that where you are actually going to prepare the cell lysate. After that the subsequent steps are going to be remain identical whether it is a prokaryotic cell, whether it is a eukaryotic cell. Now once you isolated the RNA, you are actually going to have the first question. What will be the concentration of this RNA and whether the quality of this RNA is good or not.

Just remember that when we were talking about the DNA, we have also asked the same thing. So in the next step, we are going to talk about the DNA, RNA, whether the concentration and as well as the purity. So the purity of the RNA can be detected by the same way that RNA is also going to absorb very strongly at 260 nanometer. So what you can do is you can take the absorbance at the 260 nanometer. And once you do the RNA at 260 nanometer, it is actually going to give you the values. So if there is a, you know, the pure RNA, right, if it is a pure RNA, it is actually going to have the very specific absorbance at the 260 nanometer.

If it having the protein contamination, then it is actually going to have the ratio of 260 by 280 vary from the RNA species. So then it is actually going to have the, so if it is a pure RNA, the 260 by 280 would be around 2, right. Because RNA absorbs very strongly at 260 nanometer. But if it is having the protein contamination, then it is actually going to have the level at less than 2, right.

And that is how you can be able to know the purity of the system, right. Or you can be able to have the purity of RNA. Now the next question comes is how you can be able to do the estimations. So the estimation can be done at the absorbance, right. So you can take the absorbance at 260 nanometer and you can be able to use the formula to determine the excitations or you can actually be able to use the colloidal chip method. So RNA estimation by the Orsenol method.

Remember that when we were talking about the DNA estimations, we have talking about the, we have discussed about the DPA method, right. So what is the principle? This is a general reaction for pentoses and depends upon the formation of furfural when the pentose is heated with the concentrated hydrochloric acid. Orsenol react with the

furfural in the presence of the ferric chloride as a catalyst to give the green color which can be measured at 660 nanometers.

So you are, what you are going to do is RNA. RNA is ribonucleic acid, right. Which means it is going to have the ribose as a sugar, right. Remember that for the DNA it is deoxyribose and that is why. So when you heat this RNA in the presence of the HCl, it is actually going to form the furfural, right.

And the furfural is actually going to react with the Orsenol. And it is going to give you the green color compound. Or solution. And that actually is going to have the lambda max at 665 nanometer. Okay. So this 665 nanometer. So what you can do is you can actually be able to do a calibration curve with the lambda absorbance at 665 nanometer versus the RNA, right.

So you can actually we take the different concentration of RNA and that is how it is actually going to give you the calibration curve. And then you can actually be able to run the same with the unknown samples. And then suppose this is the absorbance of the unknown samples and then you can actually be able to determine the concentration of the RNA. So what are the things you require? So you require the standard RNA solutions.

So you can require the 0.2 mg per ml in 1% per chloric acid or the buffer saline. Then you also require the Orsenol reagent. So you can dissolve the 0.1 gram of ferric chloride in 100 ml of concentrated HCl and add the 3.5 ml of 6% weight by volume Orsenol in the alcohol.

And then you also require the buffered saline. So you can actually make the NaCl and you can make the shifted buffer pH 7. So it is very simple. You do not require a lot of reagents also.

And then the procedure. So you can actually be able to pipette out the different concentration or different amount of the RNA like 0.2, 0.4, 0.6, 0.8 and 1 ml. And to a working standard to a release of labeled test tubes. Then you pipette out the 1 ml of the given sample in another test tube. Make up the volume to 1 ml in all the test tubes. Test tube with a 1 ml of distilled water serves as a blank, right? So that is the blank reaction so that you know what will be the background absorbance of the Orsenol reagent itself.

Then you add the 2 ml of Orsenol reagent to all the test tube including the test tube labeled as the blank and as well as the unknowns. Mix the content of the tube by vertexing or shaking of the tube and heat on a boiling water bath for 20 minutes. So this step and then cool the content and record the absorbent at 660 nanometer against the

black, right? Then plot the standard curve by taking the absorbance concentration of the RNA along the x axis and the absorbance at 650 via the y axis. Then from this standard curve calculate the concentration of the RNA in the given sample.

So this is the table what you are going to use. So from the standard RNA stock you are going to have the 0, 0.2, 0.4, 0.6, 0.8 and 0.1. Then you add the water so make up the volume at 1 ml. So total volume of the RNA and the water is going to be 1 ml.

So for example in this case you do not have the RNA so you are going to only take the 1 ml water. In this case you have 0.8, 0.2 so you are going to add 0.8. So total is actually going to be 1 ml, okay? And then take this table and then for unknown you are going to take the 1 ml of unknown. You can take the other values of unknown also but accordingly you are going to add the water. And then you are going to add the volume of the reagents. You are going to add the 2 ml of the Orsenol reagent which you are going to prepare with the help of the ferric chloride and HCl and Orsenol.

And then you incubate this in boiling water bath for 20 minutes then you cool down and then you are going to take the absorbance. So absorbance what you are going to get for the 0 RNA is going to be treat as the blank, right? And this has to be subtracted although you are not going to get the 0 values but you are going to subtract that value so it is going to be 0. And then you are going to have some values for other value, other concentrations and for the unknown as well. Using these values you can be able to draw a calibration curve. So what you are going to do is you are going to have the absorbance at the 665 versus the RNA concentrations, right? Which means the microgram.

And then you are going to have the standard curve. So with the help of the standard curve you can be able to determine the concentration of the unknown RNA species, right? And if you want to read more about the RNA estimations and other kinds of things you can be able to go with this Plummer's book. This is a very important, very interesting book which is dealing with the practical aspect of the biochemistry. And it is very interesting because it gives you the step by step, you know the steps how you can be able to follow and how you can be able to prepare the recipes and so on. So this is all about the RNA what we have discussed.

What we have discussed? We have discussed about the structure of the messenger RNA and the different parts of the messenger RNA and what are their functions. And then we have also discussed about how you can be able to isolate the RNA and how you can be able to verify the RNA with the help of the estimations and with the help of the purity of the RNA. So with this I would like to conclude our lecture here. Subsequent lecture we are going to discuss about the proteins which are going to be the building block. And

then we are also going to discuss about the different types of enzymes which are actually going to be participate into the molecular biology. So with this I would like to conclude my lecture here. Thank you. .